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14. ABSTRACT Given the requirement of the AR activation pathway for prostate cancer growth and progression, it is necessary to identify alternative means of targeting this pathway for the treatment of prostate cancer. The work herein has examined the role of BAF57 in its ability to activate the androgen receptor (AR). The research carried out under this proposal has fine mapped the AR binding site on BAF57 to the N-terminus (proline-rich region). Furthermore, the DBD and hinge region of AR also appear to play a significant role in the ability of BAF57 to activate AR. Together, this data presents the idea of targeting the interaction between AR and BAF57 at the N-terminus for the possibility of using this as a therapeutic for prostate cancer. Additional studies will determine the actual function of BAF57 as well as the efficacy of targeting this interaction.					
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Table of Contents

Introduction.....	4
Body.....	5-15
Key Research Accomplishments.....	16
Reportable Outcomes.....	17
Conclusions.....	18-19
References.....	20
Appendices.....	21-23

Introduction

The development and progression of prostate cancer relies on the activity of the androgen receptor (AR). AR is a hormone activated transcription factor that is necessary for normal prostate development and function. In cases where the cancer is confined to the prostate, treatment is virtually always effective. In later stages, treatment employed relies on the requirement of androgen for disease progression. Patients are subjected to hormone ablation therapy, which is generally effective. Unfortunately, this effectiveness is only temporary. After two to three years, the cancer returns and has found a means to bypass the requirement of androgen. Current research focuses on targeting the AR activation pathway, as the AR becomes re-activated and remains an essential component to the advancement of late stage prostate cancer. As a transcription factor, many additional molecules assist the AR in its ability to activate transcription. One such group is the SWI/SNF chromatin remodeling complex. SWI/SNF is composed of a core ATPase, used for remodeling chromatin to either repress or activate gene transcription, and several BRG1-associated factors (BAFs) that serve as accessory proteins to the ATPase. We found that the BAF57 subunit is an essential factor in the activation of AR. *It is our goal to determine the mechanism of BAF57 action on AR activation and examine the efficacy of targeting BAF57 as a means of treating prostate cancer.* Thus far, we have made significant progress on completing the tasks brought forth in the initial proposal. The binding region between AR and BAF57 has essentially been identified, as was the goal of task 1. Furthermore, functional studies have been utilized to confirm the importance of the AR: BAF57 interaction site.

Body

Task 1: Elucidate the necessary functional domains of BAF57 involved in regulating AR activity.

Overview of Task 1:

The goal of task 1 was to essentially determine the necessary domain(s) of BAF57 in regulating activation of the androgen receptor (AR). Based on previous studies from our lab, we identified BAF57 as a critical component of the AR activation pathway. To further identify the role of BAF57 in AR activation, we have fine-mapped the region of AR binding to BAF57. Additionally, we took the proposed task one step further to complement these studies. We identified at least two regions on AR to which BAF57 binds.

Upon completion of identifying the binding region between BAF57 and AR, functional studies were initiated. Currently, much progress is being made to examine the necessary functional domain(s) for BAF57 function in the AR activation pathway. Again, to complement these studies, we are also examining a critical domain within the AR that is required for full activation by BAF57.

Accomplishments of Task 1:

BAF57 binds to DNA binding domain (DBD) and hinge region of AR

As outlined in the initial proposal, the first task was to identify the regions of binding between BAF57 and the AR. As opposed to constructing GST fusion proteins from the BAF57 mutants, we took the approach of using AR deletion mutants already fused to GST (deletion mutants of AR are necessary as attempts of cloning full length AR into the GST expression vector have proven unsuccessful). Consequently, we used the BAF57 deletion mutants (obtained from collaborator Dr. Trevor Archer (1)) for *in vitro* transcription/translation. This approach not only allowed us to determine where AR is interacting on the BAF57 surface, but given the number of GST-AR mutant constructs in our possession, it was also possible to identify (at least in part) where BAF57 is interacting on the surface of AR. In order to use our GST-AR fusion proteins, it was necessary to narrow down the region of BAF57 binding to AR. Therefore, the first step we took was to determine whether BAF57 binds to the N- or C-terminus of AR. As seen in Figure 1, strong binding occurs between GST-BAF57 and AR 506-919 indicating a C-terminal binding site.

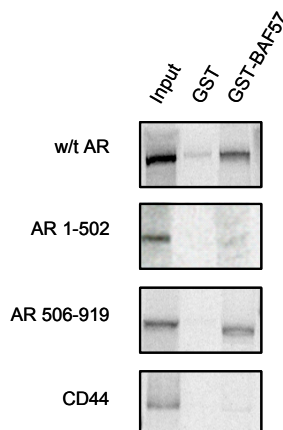


Figure 1. BAF57 bindings to C-terminus of AR. GST-BAF57 was purified and bound to glutathione agarose. Purified protein was incubated with ^[35]S-Methionine labeled wtAR, AR 1-502 (N-terminus), AR 506-919 (C-terminus), or CD44 (negative control). Binding was detected via autoradiography.

Included in the AR C-terminal region of the above construct are the well-characterized zinc finger DNA binding domain (DBD) and the hinge region. Given the significant role of these two domains in AR DNA binding and activity, we assessed the ability of BAF57 (which also harbors the ability to strongly bind DNA (2)) to bind to the AR DBD and/or hinge region. As can be seen from the GST-pulldown analysis in Figure 2, strong binding of BAF57 occurs at the DBD and hinge regions of AR.

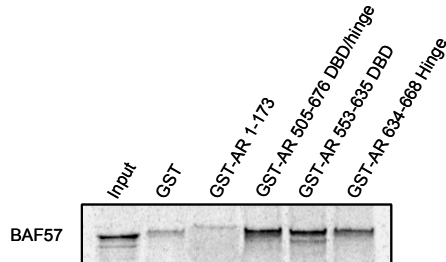


Figure 2. BAF57 binds to DBD and hinge region of AR. GST-AR fusion proteins were purified and incubated with 35 S-Methionine labeled BAF57. BAF57 binding to the GST-AR deletion mutants was detected by autoradiography.

AR hinge domain required for complete BAF57 binding and activation

To further characterize the BAF57 binding site on AR, we utilized an AR construct (AR Δ 629-636) lacking a small portion of the hinge domain. GST-BAF57 binding to this AR mutant was reduced by ~50% as determined by semi-quantitative methods (Figure 3).

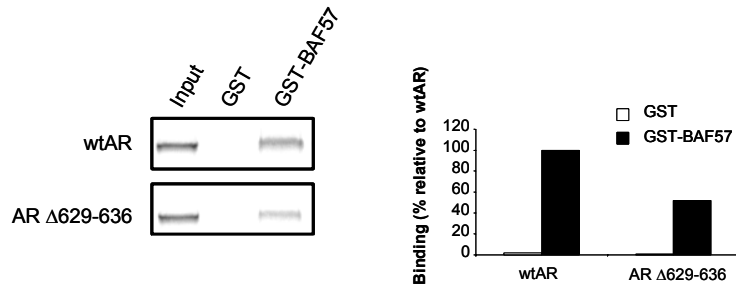


Figure 3. AR hinge domain is necessary for BAF57 binding. Purified GST or GST-BAF57 was incubated with 35 S-Methionine labeled AR or AR Δ 629-636. Binding was detected by autoradiography. GST-BAF57 binding to wtAR was set to "100."

Having established a concrete binding site for BAF57 on AR, it was then necessary to examine the ability of BAF57 to activate this AR hinge deletion mutant. Therefore, reporter analysis was carried out and AR activation by BAF57 was examined. As seen in Figure 4, the ability of BAF57 to activate the mutant AR is significantly reduced compared to the activation seen with wtAR. Together, this identifies the AR hinge region as a necessary region for the action of BAF57 in modulating AR activity.

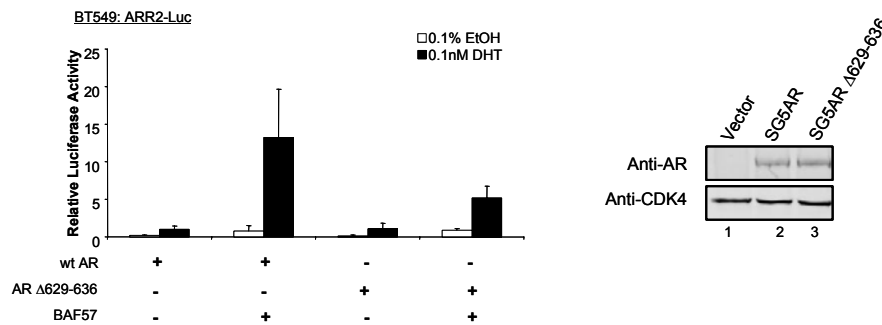
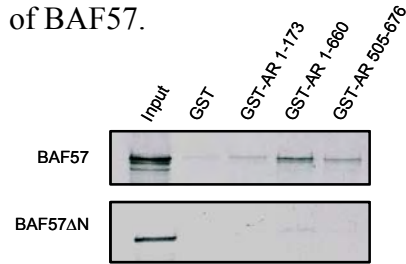


Figure 4. AR hinge region necessary for activation via BAF57. Left, BT549 cells (BAF57 null) were transfected with AR or AR Δ 629-636 and +/- BAF57. Cells were treated with 0.1nM DHT or 0.1% EtOH as indicated and AR activity was measured via the ARR2 reporter tagged to luciferase. Right, Western blot analysis showing expression of AR and mutant AR.

AR binds to proline-rich region of BAF57

Having established the DBD and hinge domains of AR as critical binding sites for BAF57, it was then possible to proceed to identify the region of BAF57 that is necessary for AR binding. Initially, several GST-AR fusion proteins were utilized to assess BAF57 binding. Additionally, an N-terminal deletion mutant of BAF57, BAF57 Δ N, was examined in its ability to bind AR. Interestingly, as seen in Figure 5, BAF57 Δ N is unable to bind to AR as compared to full length BAF57. This finding enabled us to focus the remainder of our binding studies within the N-terminus of BAF57.

Figure 5. AR is unable to bind to BAF57 N-terminal deletion mutant. GST-pulldown analysis was carried out using GST or the GST-AR fusion proteins as indicated. Binding to BAF57 or BAF57 Δ N is shown.



In order to demonstrate sufficiency of AR binding to the N-terminus of BAF57, I constructed a BAF57 deletion mutant containing only the N-terminus (amino acids 1-145). Upon successful cloning of the BAF57 1-145 construct, GST-pulldown analysis was carried out and as expected, significant binding to AR was observed (Figure 6).

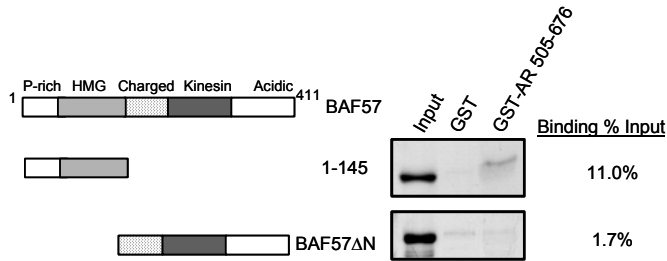


Figure 6. BAF57 N-terminus is sufficient for AR binding. GST-pulldown analysis was carried out using GST or GST-AR 505-676 as indicated. Binding to BAF57 1-145 or BAF57 Δ N is shown.

After identifying the first 145 amino acids of BAF57 as containing the primary binding site(s) for AR, the binding site was further refined so as to accomplish the overall goal of the study. After obtaining two BAF57 deletion mutants from our collaborator, GST-pulldown analysis was performed with GST or GST-AR 505-676 and full length BAF57, BAF57 Δ N, BAF57 Δ PR, or BAF57 Δ HMG. As depicted in Figure 7, AR binds strongly to the proline-rich domain of BAF57.

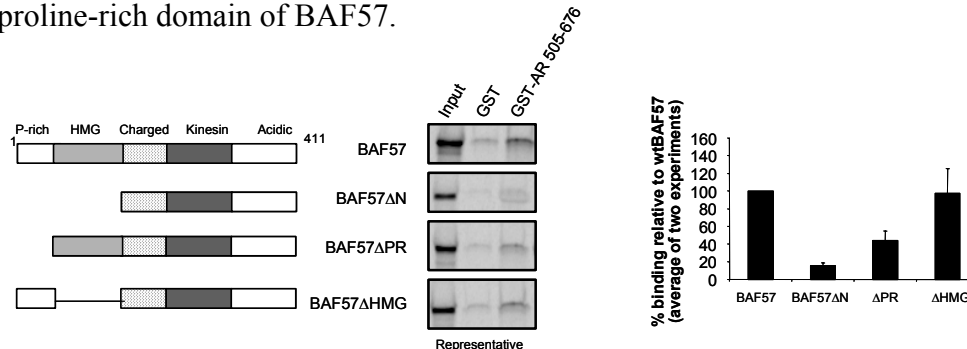
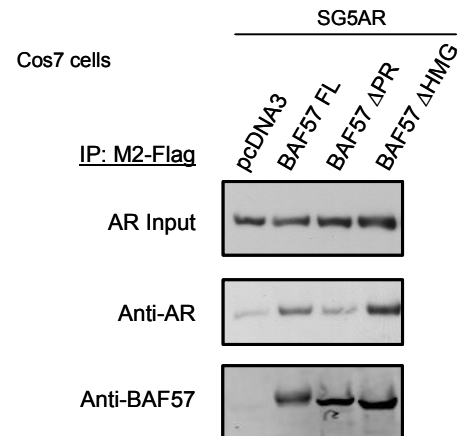


Figure 7. AR binds proline-rich region of BAF57. GST-pulldown analysis was carried out as above, and binding from two separate experiments was quantitated with AR binding to full length BAF57 set at “100.”

Additionally, binding was also assessed using a cell culture model, wherein Cos7 cells were transfected with full length AR and the BAF57 mutants listed. Co-immunoprecipitation analysis was carried out and levels of AR binding were assessed through immunoblotting (Figure 8). Together, these data indicate that AR binds strongly to the proline-rich domain of BAF57.

Figure 8. AR binds BAF57 proline-rich domain in cultured cells. Cos7 cells were transfected with AR, H2B-GFP (transfection efficiency), and pcDNA3 (empty vector) or the indicated flag-tagged BAF57 constructs. Following transfection, cells were harvested and immunoprecipitation was carried out. BAF57 protein was IP'd using M2 flag resin, and AR binding was analyzed by immunoblotting. Transfected BAF57 levels are also shown.



BAF57 N-terminus blocks full-length activation of AR

In accordance with task 1, the functional impact of BAF57 was to be examined on its ability to activate AR. These experiments were carried out utilizing the BAF57 1-145 N-terminal construct. As demonstrated in the above section, AR clearly binds to the N-terminus of BAF57. Therefore, we tested the ability of BAF57 1-145 to inhibit the activity of AR elicited by wtBAF57, as this mutant is unlikely to maintain SWI/SNF association given the ability of BAF57ΔN to assemble into the SWI/SNF complex (3). Figure 9 demonstrates that increasing concentrations of BAF57 1-145 are able to significantly reduce AR activity.

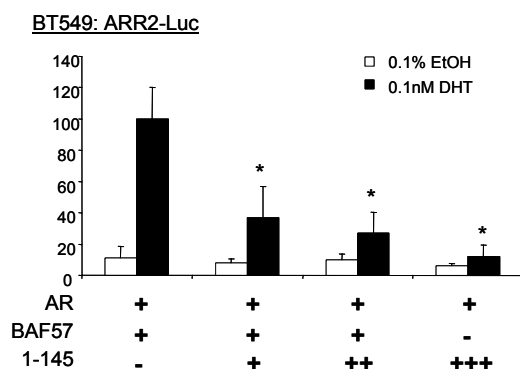


Figure 9. BAF57 1-145 inhibits wtBAF57 activation of AR. BT549 cells were transfected with AR, BAF57, or BAF57 1-145 as indicated. Cells were treated with 0.1nM DHT or 0.1% EtOH as indicated and AR activity was measured via the ARR2 reporter tagged to luciferase. AR + BAF57 + DHT is set to "100." Asterisk indicates statistical significance $p < 0.05$ based on the Student t-test.

To complement the above transcriptional studies, the N-terminus of BAF57 was tested in its ability to block endogenous AR activity. To carry out these studies, the LNCaP prostatic adenocarcinoma cell line was employed. LNCaP cells harbor endogenous AR activity, which can be monitored through the expression of the prostate specific antigen (PSA). Expression of PSA was examined upon transfection of these cells with either vector (negative control), dominant negative AR (positive control), or BAF57 1-145. As demonstrated in Figure 10, BAF57 significantly attenuates PSA expression similar to the dnAR.

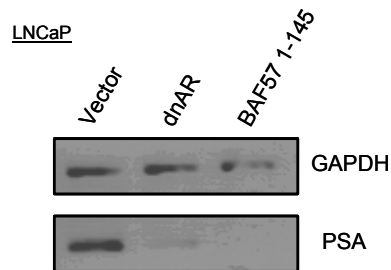


Figure 10. BAF57 1-145 inhibits PSA expression. LNCaP cells were transfected with H2B-GFP (transfection control) and the indicated expression plasmids. RNA was isolated, reverse transcriptase PCR was performed, and PSA was amplified. GAPDH was amplified a loading control.

Summary of Task 1 Accomplishments

As the goal of task 1 was to determine the necessary functional domain(s) of BAF57 in regulating AR activity, we first set out to determine the site of interaction between BAF57 and AR. Although not part of the initial proposal, it was first necessary to identify the point of BAF57 interaction on AR. Using several GST-AR deletion mutants, we were able to narrow down the binding of BAF57 to the DBD and hinge region of AR. However, our studies have not ruled out the possibility of an interaction site for BAF57 within the ligand binding domain of AR. Taking this route also highlighted the requirement of the AR hinge domain for activation by BAF57. In addition to allowing us to continue with identifying the AR interaction site(s) on BAF57, narrowing down where BAF57 interacts with the AR surface was certainly an added and exciting bonus. Given the strong ability of BAF57 to bind the DBD and hinge region of AR, the GST-AR 505-676 fusion protein containing both the DBD and hinge region was utilized to determine which region(s) of BAF57 are necessary for interacting with AR. GST-pulldown analysis confirmed by co-immunoprecipitation demonstrate that the N-terminal proline-rich region of BAF57 is the primary point of AR interaction. Furthermore, the activation of AR, elicited through BAF57, is compromised when the N-terminal BAF57 1-145 region is introduced.

Task 2: Delineate the molecular mechanism of BAF57 action on AR function, thereby establishing BAF57 as a putative therapeutic target.

Overview of Task 2:

Previously, our lab has shown the recruitment of BAF57 to the PSA enhancer element in response to ligand. Furthermore, we have clearly identified BAF57 as a critical factor in regulating AR activity (4). However, the mechanism of BAF57 action on AR is undefined. As task 1 identified the region of BAF57 and AR interaction, task 2 aims to examine the mechanism of how BAF57 elicits its function on modulating AR activity.

We have made significant progress on determining the function of BAF57 in activation of AR.

Accomplishments of Task 2:

BAF57 is intermittently recruited to the PSA locus along with Pol II

Evidence in the literature has indicated that AR is constantly recruited to androgen responsive elements (AREs) within the PSA gene over a period of time up to 16h (5). In accordance with this finding, we show constant occupancy of AR over a five hour range of DHT stimulation at the PSA enhancer region as depicted in Fig. 11A. Interestingly, examination of BAF57 recruitment in response to DHT over this same time period demonstrated recruitment of BAF57 at one and two hours of DHT treatment, but subsequent loss of BAF57 occupancy past three hours (B) (it should be noted that this experiment was performed several times and this data is representative of the findings). Additionally, examination of RNA pol II (indicative of active transcription) revealed strong recruitment at the early time points, and a decrease in occupancy at the fourth and fifth hours of stimulation (C).

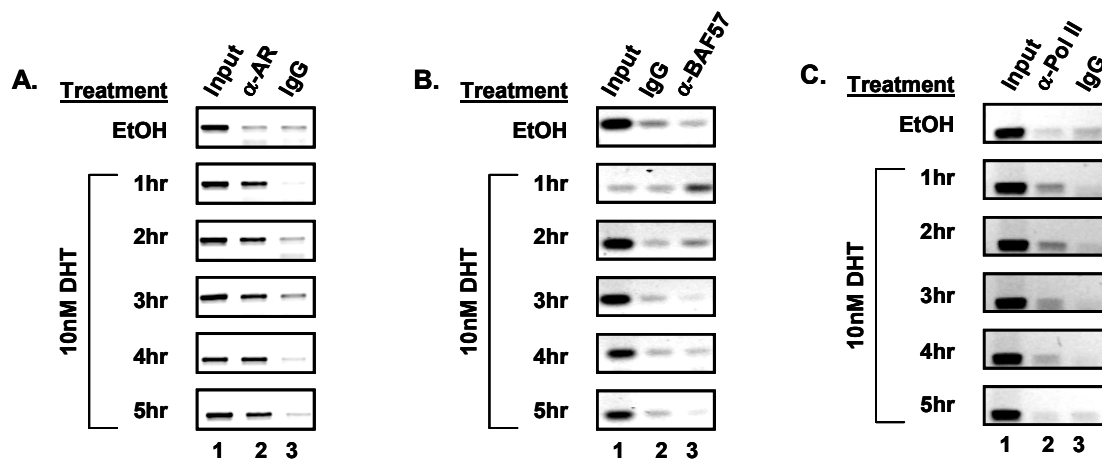


Figure 11. BAF57 is temporally recruited to the PSA enhancer element. LNCaP derived prostate cancer cells (LB1-Luc) were cultured in steroid-free medium for 3 days and then treated with either 0.1% EtOH or 10nM DHT for the indicated time. Cells were harvested for chromatin immunoprecipitation (ChIP) analyses and antibodies to AR (A), BAF57 (B), Pol II (C), or preimmune serum (IgG) were utilized to examine recruitment to the PSA enhancer region. At least two independent experiments were performed and a representation is depicted.

BAF57 N-terminus disrupts BAF57 and AR stabilization on the PSA enhancer element.

Having identified the N-terminal region of BAF57 as the primary interaction site for AR and establishing its ability to suppress AR target gene transcription, we tested the ability

of this peptide to disrupt SWI/SNF and AR recruitment/stabilization at the PSA enhancer element. LNCaP prostate cancer cells were transfected with vector control or BAF57 1-145 and H2B-GFP to monitor transfection. Following transfection, cells were stimulated for 1hr with DHT. ChIP analysis was carried out to determine BAF57 and AR recruitment. As shown, BAF57 and AR were both strongly recruited in the presence of vector transfection. However, recruitment was significantly attenuated upon transfection of the BAF57 1-145 peptide. Together, these results indicate that we can successfully disrupt SWI/SNF mediated AR function by disrupting a primary link between them, BAF57. Furthermore, AR also appears dependent on BAF57 function for stabilization at the PSA enhancer.

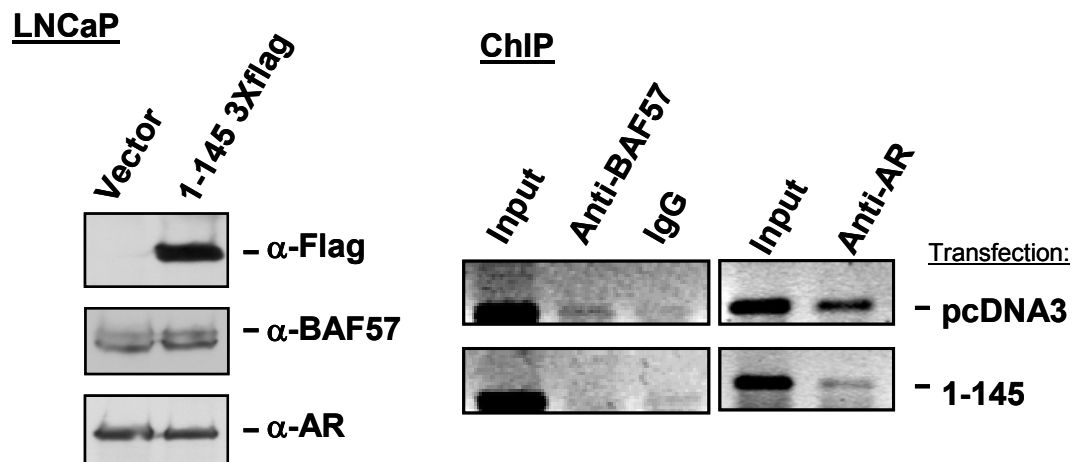


Figure 12. BAF57 N-terminus reduces BAF57 and AR residence at AREs. LNCaP cells were transfected with 2μg H2B-GFP and 8μg of either pcDNA3 or pcDNA3-BAF57 1-145 3Xflag. Post transfection, medium was changed to steroid free conditions for 48h. Cells were stimulated with 10nM DHT for 1h and subjected to immunoblot analysis for Flag, BAF57, or AR (left panel) or ChIP analysis for BAF57 and AR recruitment to the PSA enhancer region (right panels).

Summary of Task 2 Accomplishments

The above data indicates a possible role for BAF57 in stabilizing or recruiting AR to the PSA enhancer element. The remainder of task 2 must be carried out however, to draw any definite conclusions. Determining the role of BAF57 in the recruitment of SWI/SNF to AREs, in addition to examining a possible role for BAF57 in nucleosomal repositioning are two important tasks that will assist in determining the function of BAF57 in regulating AR activation.

Task 3: Examine the requirement of BAF57 for proliferation in early and late stage prostate cancer.

Overview of Task 3:

The primary drive behind task 3 is to determine the significance and ability to target the BAF57-AR interaction *in vivo*. Although not yet carried out fully, initial studies have begun examining expression in advanced prostate cancer samples. Results below show

expression of BAF57 in all prostate cancer cell line and tissue examined implicating BAF57 as a good choice for therapeutic targeting.

Accomplishments of Task 3:

BAF57 is expressed in all prostate cancer cell lines and metastatic tissue examined.

First, western blot analysis has been carried out in a multitude of prostate cancer cell lines as shown in the initial proposal. Shown below (Figure 12), BAF57 is strongly expressed in all prostate cancer cell lines examined. Additionally, immunohistochemistry data shows strong expression of BAF57 in two prostate cancer metastases (lymph node) similar to expression of AR. To gain a more comprehensive appreciation of BAF57 expression in prostate cancer, we performed a tissue microarray on several stages of prostate cancer. As depicted, BAF57 is highly expressed in all specimens examined, indicating that BAF57 is maintained in prostate cancer. This suggests that BAF57 is in fact a valid target for the treatment of prostate cancer, even metastatic disease.

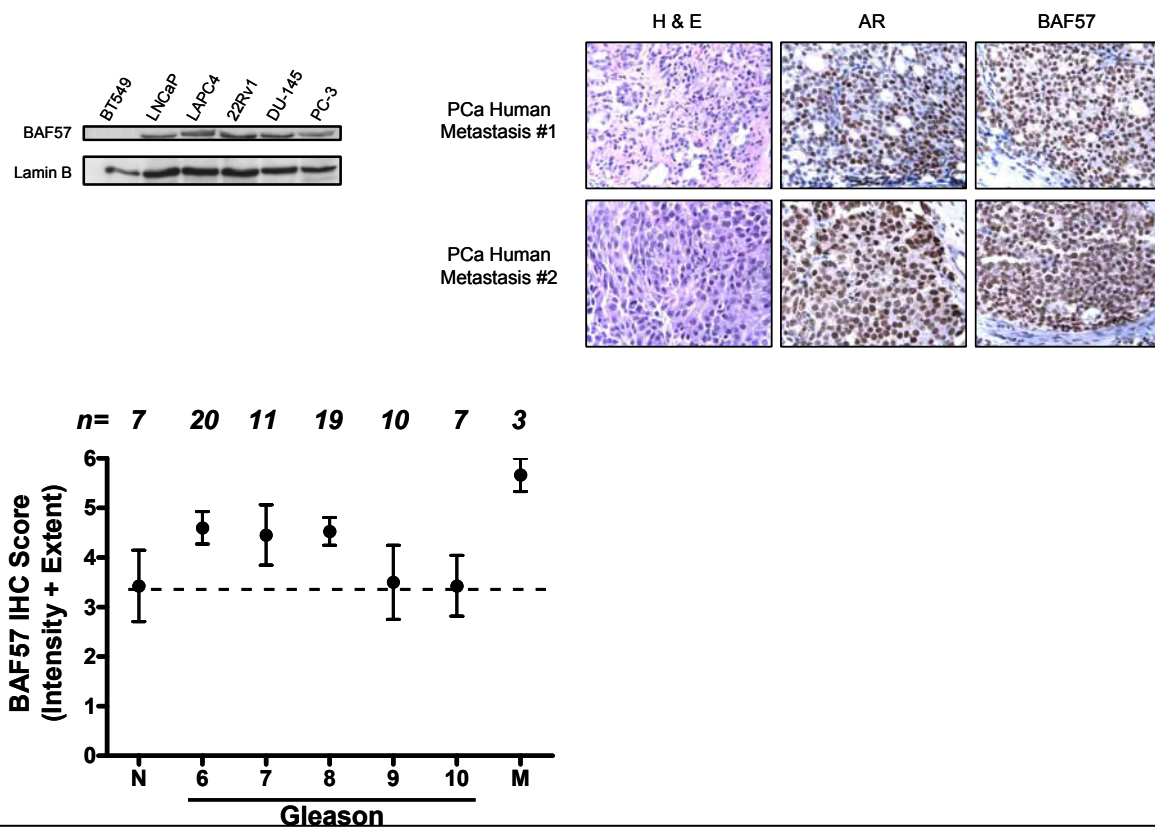


Figure 13. BAF57 is expressed in prostate cancer tissue. Left panel, Western analysis showing expression in all prostate cancer cell lines tested. BT549 cells were used as the negative control. Right panel, Immunohistochemistry analysis demonstrating expression of BAF57 in two prostate cancer metastases. Comparatively, AR is similarly robustly expressed. Bottom panel, IHC for BAF57 expression was observed in all PCa grades tested.

Given that we have identified the primary region of BAF57 that interacts with AR, and that this region attenuates AR activity, we next examined the ability of this motif to

disrupt AR dependent proliferation. LNCaP cells were transfected with vector, dnAR (positive control), or BAF57 1-145 and H2B-GFP to monitor transfection. BrdU incorporation was monitored. As seen, similar to the dnAR positive control, BAF57 1-145 was able to significantly attenuate LNCaP prostate cancer cell proliferation, indicating that disruption of the AR-BAF57 interaction site through overexpression of the BAF57 binding peptide may be effectively developed as a prostate cancer therapeutic.

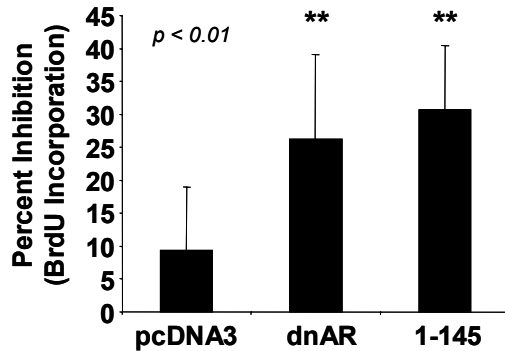


Figure 14. Disruption of prostate cancer cell growth through AR-BAF57 disruption. LNCaP cells were transfected with H2B-GFP and pcDNA3, pSG5AR Δ 46-408 (dnAR), or pcDNA3-BAF57 1-145 3Xflag. 48h post transfection, bromodeoxyuridine (BrdU) was added for a 16h pulse, cells were fixed, and staining for BrdU incorporation was performed. Results from at least three independent experiments are plotted as percent inhibition of BrdU incorporation over vector control. ** = $p < 0.01$.

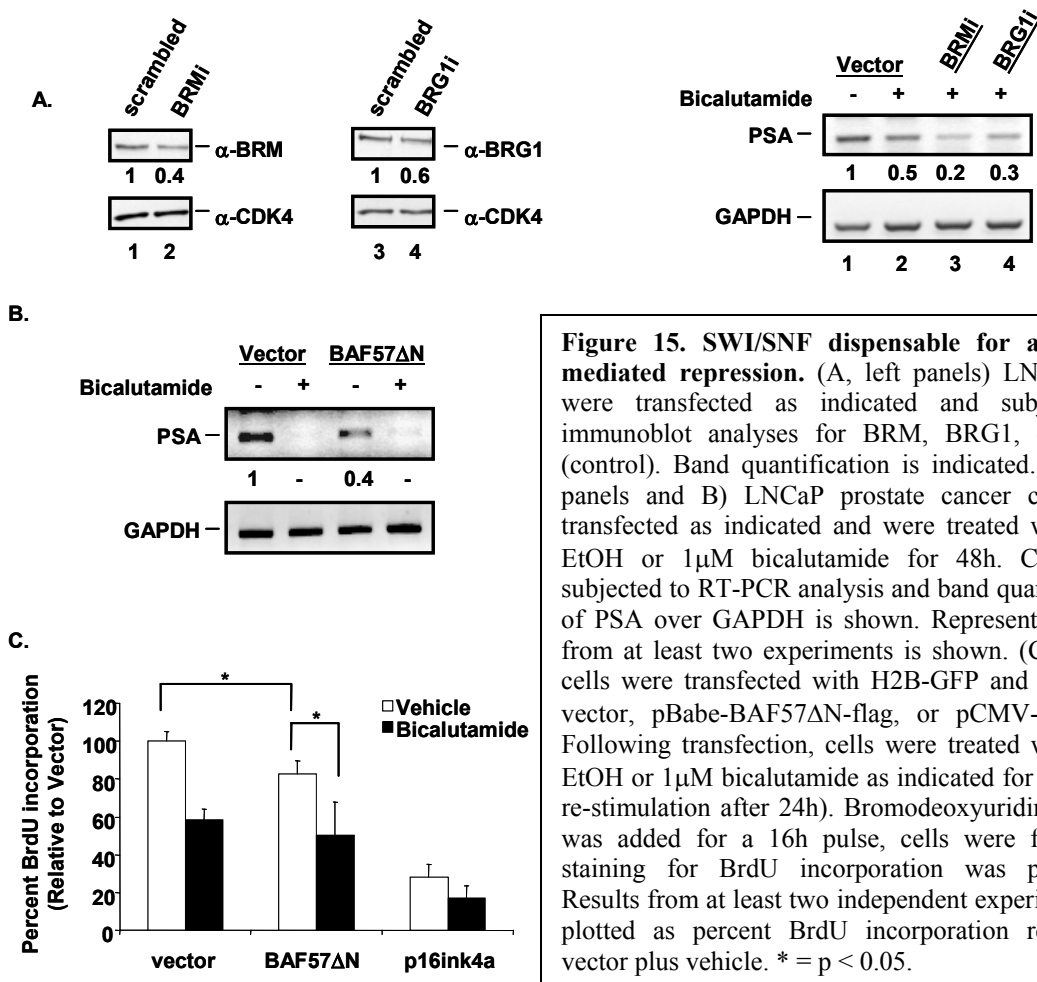


Figure 15. SWI/SNF dispensable for antagonist mediated repression. (A, left panels) LNCaP cells were transfected as indicated and subjected to immunoblot analyses for BRM, BRG1, or CDK4 (control). Band quantification is indicated. (A, right panels and B) LNCaP prostate cancer cells were transfected as indicated and were treated with 0.1% EtOH or 1μM bicalutamide for 48h. Cells were subjected to RT-PCR analysis and band quantification of PSA over GAPDH is shown. Representative data from at least two experiments is shown. (C) LNCaP cells were transfected with H2B-GFP and pcDNA3-vector, pBabe-BAF57ΔN-flag, or pCMV-p16ink4a. Following transfection, cells were treated with 0.1% EtOH or 1μM bicalutamide as indicated for 48h (with re-stimulation after 24h). Bromodeoxyuridine (BrdU) was added for a 16h pulse, cells were fixed, and staining for BrdU incorporation was performed. Results from at least two independent experiments are plotted as percent BrdU incorporation relative to vector plus vehicle. * = $p < 0.05$.

SWI/SNF ATPases and BAF57 are

not required for bicalutamide mediated repression of AR As SWI/SNF is necessary for AR function it could be hypothesized that blocking SWI/SNF recruitment to AR target genes may provide a new mechanism to suppress AR activity in cancer. However, the importance of SWI/SNF for the response to AR antagonist used in therapy has not yet been assessed. This point is important given that common treatment for prostate cancer includes administration of AR antagonist in conjunction with other therapies. To address this, the impact of SWI/SNF was initially examined after knockdown of the central ATPases, BRM (60% knockdown) or BRG1 (40% knockdown) (Fig. 15A, left panel). LNCaP cells were transfected with H2B-GFP and either vector, BRMi, or BRG1i and treated with bicalutamide as indicated (Fig. 15, right panel). As expected, the AR target gene PSA was highly expressed in the vector transfected, vehicle treated sample (lane 1). Upon treatment with bicalutamide, relative expression of PSA was reduced by 49% as compared to vehicle treated (lane 2 vs. lane 1). Additionally, knockdown of BRM with the addition of bicalutamide resulted in a greater reduction in PSA expression as compared to bicalutamide treatment alone (79%, lane 3 vs. lane 1). BRG1 depletion with bicalutamide treatment initiated a 69% drop in PSA expression compared to vehicle

treated vector (lane 4 vs. lane 1). Thus, these data indicate that SWI/SNF function is likely dispensable for the response to AR antagonists.

To validate the above finding, the impact of BAF57 was examined (B,C). Experiments were performed as above after introduction of dominant negative BAF57 (BAF57ΔN), as attempts to establish significant ablation of BAF57 in AR positive prostate cancer cells have proven unsuccessful. As expected, vehicle treatment and vector transfection demonstrated robust PSA mRNA expression, whereas the addition of bicalutamide caused a complete loss in expression (lane 1 and lane 2, respectively). Furthermore, inhibition of BAF57 function upon transfection of BAF57ΔN showed a 63% reduction in PSA expression upon vehicle treatment compared to vector transfection (lane 3 vs. lane 1). Upon treatment with bicalutamide, disruption of BAF57 function provided no change in the ability of bicalutamide to repress AR activity. Thus, similar to what was shown after knockdown of BRM or BRG1, BAF57 activity was not necessary for bicalutamide-mediated repression of AR activity. Additionally, the requirement of BAF57 for bicalutamide-mediated suppression of AR dependent prostate cancer proliferation was examined (C). Upon transfection with control vector, BrdU incorporation analyses revealed a 42% reduction in LNCaP prostate cancer cell proliferation in response to treatment with bicalutamide compared to vehicle treatment. Consistent with previous reports, the cell cycle inhibitor p16ink4a was utilized as the positive control, and demonstrated significant attenuation (72% for vehicle treated; 83% for bicalutamide treated) of LNCaP cell proliferation compared to vector control. Inhibition of BAF57 function demonstrated a 17% reduction in LNCaP cell proliferation upon vehicle treatment. Furthermore, bicalutamide treatment remained equally as effective at inhibiting prostate cancer cell proliferation upon BAF57ΔN transfection as compared to the vector transfected cells with the same treatment. Together, these data indicate that disruption of SWI/SNF or BAF57 alone does not compromise the response to AR antagonist. Given the established requirement of BAF57 for the response to AR agonists, these observations suggest that the SWI/SNF-AR interaction could be developed as a therapeutic target.

Summary of Task 3 Accomplishments

Work is clearly progressing quite well on task three and should be wrapped up in the final year. The advances within the past year will make significant impact on the prostate cancer field. Combined, these data indicate that it is possible to disrupt the BAF57-AR interaction by means of a BAF57 peptide that specifically binds to the AR, however, this region does not interact with the SWI/SNF complex indicating that SWI/SNF may not be able to be recruited to AR target genes. Additionally, the discovery that SWI/SNF may be dispensable for AR antagonist function highlights the potential for targeting BAF57 as an adjuvant therapy with antagonist administration.

Key Research Accomplishments

- Our studies indicate that BAF57 binds to the AR DBD as well as the hinge region. Furthermore, AR activity elicited by BAF57 requires the AR hinge domain for full activation. This finding complements the examination of the BAF57 regions extremely well, and provides one more avenue to explore in determining the signaling between BAF57 and AR.
- We demonstrate that the N-terminal region (primarily the proline-rich region) of BAF57 is necessary and sufficient for AR binding. Identification of the binding region gives tremendous rationale for investigating this region further for its functional potential, which was examined as described below.
- To complement the binding studies, we also show that expression of the N-terminus of BAF57 is able to inhibit the ability of full length BAF57 to activate the AR. This finding indicates the N-terminus of BAF57 as a critical player in activating the AR.
- The recruitment of BAF57 to AREs was shown to be an early response similar to AR coactivators, with subsequent loss of recruitment.
- We have shown that BAF57 may confer AR stabilization on the PSA enhancer region, and this may be occurring through the N-terminus as indicated above.
- Given that the ultimate goal is to prevent prostate cancer proliferation, we determined that expression of the BAF57 binding peptide is able to significantly attenuate prostate cancer cell proliferation.
- Additional studies reveal that SWI/SNF is dispensable for antagonist mediated repression of AR.
- Finally, we have found robust expression of BAF57 in all human prostate cancer grades examined including metastatic prostate cancer, lending to the ability of actually targeting this protein in the treatment of advanced prostate cancer.

Reportable Outcomes

Manuscripts: One is submitted to Cancer Research

Abstracts/Presentations:

1. Link et. al. Keystone Symposia on Nuclear Receptors. Banff, Alberta, Canada. March 2006 Poster Presentation.
2. Link et. al. Endocrine Society Annual Meeting. Boston, MA. June 2006 Poster Presentation.
3. Link et. al. University of Cincinnati Cell and Cancer Biology Seminar. Cincinnati, OH. November 2006. Oral Presentation.
4. Link et. al. AACR Innovations in Prostate Cancer Research. San Francisco, CA. December 2006 Poster Presentation (AFLAC scholar-in-training travel award).
5. Link et. al. IMPACT meeting. Atlanta, GA. Sept. 2007 Poster Presentation

Patents: None

Degrees obtained supported by this award: Ph.D. Dec. 12, 2007

Development of cell lines: N/A

Informatics: None

Funding: None applied for

Employment or research opportunities: Postdoctoral position

Conclusion

A global view of the research at hand portrays a necessity in identifying novel approaches for the treatment of prostate cancer. Therefore, much of the current research is aimed at deciphering the mechanism of androgen receptor signaling in hopes of identifying a potential target for the treatment of prostate cancer. Much progress has been made in regards to determining the role of BAF57 in AR activation. As outlined in the proposal, the study includes 1) determining the necessary functional domains of BAF57 involved in regulating AR activity, 2) identifying the molecular mechanism of BAF57 action on AR function, thereby establishing BAF57 as a putative therapeutic target, and 3) examining the requirement of BAF57 for proliferation in early and late stage prostate cancer.

Our progress has been on par with the statement of work. Our studies have identified at least one region of the AR necessary for binding (DBD/hinge) and activation (hinge) by BAF57. Furthermore, the AR binding domain within BAF57 has emerged as the N-terminal proline-rich domain. It also appears that BAF57 is also able to elicit its primary function through this N-terminus, as overexpression appears to inhibit full length BAF57 activation of AR.

In addition to the completion of task 1, work has essentially been completed for task 2. The mechanism of BAF57 action were further defined through demonstration of early recruitment of BAF57 with subsequent displacement at the PSA locus. Additionally, a potential role for BAF57 has arisen in modulating AR recruitment and/or stability on the PSA enhancer element. Furthermore, we demonstrate disruption of the BAF57 subunit upon transfection of the BAF57 peptide necessary for interaction with AR. Task 3 has had significant progress as well. BAF57 expression has been confirmed in all stages of prostate cancer, even late stage human metastatic prostate cancer. Furthermore, SWI/SNF was shown to be dispensable for antagonist mediated repression of AR, thus indicating potential dual mechanisms of targeting the AR in prostate cancer treatment.

Changes to future studies:

Initial reporter analysis in BT549 cells with the BAF57 mutants have been inconsistent, therefore we have established alternative means (as outlined in task 1 accomplishments) of examining the impact of the N-terminus of BAF57 as this was established as the primary AR interaction site. In addition to the above experiments, the ability of the BAF57 1-145 mutant to inhibit prostate cancer cell proliferation is also being examined. More consistent reporter analysis will also be sought.

Currently, plans for the remainder of the study will be carried out as outlined in the initial proposal pending no complications.

So What?

The outcomes of the above mentioned research have quite an impact on elucidating the signaling pathway of the AR and the involvement of BAF57. Given the importance of BAF57 in AR activation and proliferation of prostate cancer cells (4), the identification of the interaction surfaces between BAF57 and AR is critical. Having identified the potential interaction surfaces, we have extended these studies to examine a means of

disrupting this interaction with the anticipation of using this disruption as a therapeutic for the treatment of prostate cancer. Expression of BAF57 in human prostate cancer further confers the potential to target this SWI/SNF subunit in the treatment of prostate cancer. Inhibition of prostate cancer proliferation as demonstrated in the LNCaP cell line by the BAF57 peptide identify this region as a potential mechanism for targeting the AR-BAF57 interface and ultimately a prostate cancer therapeutic. Together, completion of this proposal will result in a detailed understanding of the SWI/SNF complex (specifically BAF57) in its ability to modulate AR activity. This understanding, in turn, will result in determining the efficacy and potential mechanisms for actually targeting BAF57 as a possible prostate cancer therapeutic.

References:

1. J. Chen, T. K. Archer, *Mol Cell Biol* **25**, 9016 (Oct, 2005).
2. W. Wang *et al.*, *Proc Natl Acad Sci U S A* **95**, 492 (Jan 20, 1998).
3. T. H. Chi *et al.*, *Nature* **418**, 195 (Jul 11, 2002).
4. K. A. Link *et al.*, *Mol Cell Biol* **25**, 2200 (Mar, 2005).
5. Q. Wang, J. S. Carroll, M. Brown, *Mol Cell* **19**, 631 (Sep 2, 2005).

Appendices

Prostate cancer training program:

- I have attended a prostate cancer working group that is held monthly, and had the opportunity to present my work at this venue.
- Our lab holds weekly lab meetings to discuss our recent findings
- We hold weekly journal club wherein a recent prostate cancer publication is discussed.
- I attended the IMPACT prostate cancer meeting sponsored by this funding support.

Kevin A. Link
CV

ACADEMIC/PROFESSIONAL PREPARATION:

Graduate Student, University of Cincinnati College of Medicine, Department of Cell and Molecular Biology, 2002-2007
B.S. in Molecular Biology, College of Mt. St. Joseph, Cincinnati, OH, 2002
Research Assistant, Wood Hudson Cancer Research Laboratory, Newport, KY, 2000-2002

SKILLS AND PARTICIPATION THROUGH GRADUATE SCHOOL

Molecular techniques, including: PCR, cloning, cell culture, real time-PCR, pull-down assays, reporter assays, immunoblotting, protein purification
Trained several students within the Knudsen laboratory throughout graduate school
Graduate course work training: scientific writing, functional genomics, molecular genetics, advanced molecular genetics, cell biology, cancer biology, biochemistry
Student representative for Cell and Molecular Biology Admissions Committee 2005
Student representative for Cell and Molecular Biology Graduate Committee 2004
Recruitment Committee member 2004-2005

PRESENTATIONS

DOD sponsored IMPACT meeting, Atlanta, GA Sept. 2007
(poster presentation)
AACR, Innovations in Prostate Cancer Research, San Francisco, CA Dec. 2006
(poster presentation)
Cell and Cancer Biology Seminar Series, University of Cincinnati Nov. 2006
(oral presentation)
The Endocrine Society Annual Meeting, Boston, MA June 2006
(poster presentation)
Keystone Symposia, Nuclear Receptors: Steroid Sisters, Banff, Alberta, Canada Mar. 2006 (poster presentation)
Great Lakes Nuclear Receptor Conference, Madison, WI Oct. 2005
(poster presentation)
AACR Edward A. Smuckler Memorial Pathobiology of Cancer Workshop Snowmass, CO July 2005 (poster presentation)
AACR, Basic, Translational, and Clinical Advances in Prostate Cancer, Bonita Springs, FL Nov. 2004 (poster presentation)
Midwest Regional Molecular Endocrinology Conference, Indianapolis, IN May 2004
(oral presentation)
Keystone Symposia, Nuclear Receptors: Steroid Sisters, Keystone, CO Mar. 2004
(Hot topics oral presentation and poster presentation)
Jensen Symposium, University of Cincinnati, Cincinnati, OH Dec. 2003
(poster presentation)

PUBLICATIONS/ABSTRACTS:

- Link, K. A.**, Comstock, C., Balasubramaniam, S., Powers, N., Cao, K., Haelens, A., Claessens, F., Revelo, M., Knudsen, K. E. (2007) Targeting the BAF57 SWI/SNF subunit in prostate cancer: a novel platform to control AR activity. Submitted to Cancer Research, presented at IMPACT meeting.
- Link, K. A.**, Burd, C., Williams, E., Marshall, T., Rosson, G., Henry, E., Weissman, B., Knudsen, K. E. (2005) BAF57 governs androgen receptor action and androgen-dependent proliferation through SWI/SNF. MCB 2005 March
- Marshall, T., **Link, K. A.**, Petre-Draviam, C., and Knudsen, K. E. (2003) Differential requirement of SWI/SNF for androgen receptor activity. J Biol Chem. 2003 May 29

HONORS AND AWARDS:

- AACR- AFLAC Scholar-In-Training Award “Innovations in Prostate Cancer Research” Dec. 2006
- University of Cincinnati College of Medicine, Graduate Student Research Forum, First Place (poster presentation) Nov. 2005
- DOD Prostate Cancer Research Program Predoctoral Traineeship Award (**W81XWH-06-1-0098**), Nov. 2005-Nov. 2007
- Accepted for AACR Edward A. Smuckler Memorial Pathobiology of Cancer Workshop, Snowmass, CO July 2005
- Albert J. Ryan Fellowship recipient, 2005
- AACR- AFLAC Scholar-In-Training Award “Basic, Translational, and Clinical Advances in Prostate Cancer” Nov. 2004
- Keystone Symposia Scholarship winner Mar. 2004
- Supported by NIEHS training grant 2 T32 ES07250-16, 2003-2005